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ESTIMATING NEAR-INFRARED LEAF REFLECTANCE FROM LEAF STRUCTURAL CHARACTERISTICS[1]

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The relationship between near-infrared reflectance at 800 nm (NIRR) from leaves and characteristics of leaf structure known to affect photosynthesis was investigated in 48 species of alpine angiosperms. This wavelength was selected to discriminate the effects of leaf structure vs. chemical or water content on leaf reflectance. A quantitative model was first constructed correlating NIRR with leaf structural characteristics for six species, and then validated using all 48 species. Among the structural characteristics tested in the reflectance model were leaf trichome density, the presence or absence of both leaf bicoloration and a thick leaf cuticle (>1 μm), leaf thickness, the ratio of palisade mesophyll to spongy mesophyll thickness (PM/SM), the proportion of the mesophyll occupied by intercellular air spaces (%IAS), and the ratio of mesophyll cell surface area exposed to IAS (A_{mes}) per unit leaf surface area (A), or A_{mes}/A. Multiple regression analysis showed that measured NIRR was highly correlated with A_{mes}/A, leaf bicoloration, and the presence of a thick leaf cuticle ($r^2 = 0.93$). In contrast, correlations between NIRR and leaf trichome density, leaf thickness, the PM/SM ratio, or %IAS were relatively weak ($r^2 < 0.25$). A model incorporating A_{mes}/A, leaf bicoloration, and cuticle thickness predicted NIRR accurately for 48 species ($r^2 = 0.43$; $P < 0.01$) and may be useful for linking remotely sensed data to plant structure and function.

Key words: A_{mes}/A; bicoloration; leaf structure; mesophyll; near-infrared; reflectance.

The optical properties of leaves have been shown to be correlated with their photosynthetic performance (Vogelmann, 1993; Smith et al., 1997) and thermal energy budgets (Gates, 1976; Ehleringer and Mooney, 1978). Moreover, an understanding of the leaf structural components that influence leaf reflectance is important for interpreting remotely sensed data, such as in the identification of plant functional types (Knipling, 1970). Leaf reflectance in the near-infrared region (NIR; 750–1350 nm) is affected primarily by leaf structure, whereas reflectance in the visible region (400–700 nm) is determined mostly by photosynthetic pigments, and reflectance in the middle-infrared region (1350–2500 nm) by water content (Gates et al., 1965). At the transition from red to NIR wavelengths, leaf reflectance greatly increases, producing a distinct spectral feature referred to as the red edge. The positioning of this edge has been correlated to chlorophyll content, plant phenological stages, as well as plant stress (Miller et al., 1991; Carter, 1993; Vogelmann, Rock, and Moss, 1993; Gitelson, Merzlyak, and Lichtenthaler, 1996). In contrast, analysis of leaf reflectance within the NIR region can be used to evaluate the effects of leaf structural properties on reflectance, as opposed to leaf chemical constituents such as chlorophyll and water (Gates, 1970; Hunt, Rock, and Nobel, 1987; Hunt and Rock, 1989; Curran et al., 1992).

Many characteristics of leaf structure may contribute to the reflectance of NIR radiation from leaves. Inside a leaf, light is scattered at the interfaces of cell walls and intercellular air spaces (IAS), due to a large change in the refractive index from 1.00 to 1.33, respectively (Willstätter and Stoll, 1913, as cited in Gausman, Allen, and Cardenas, 1969). Near-infrared reflectance from leaves has been demonstrated in previous studies to be particularly influenced by the ratio of mesophyll cell surface area (A_{mes}) exposed to intercellular air spaces (IAS) expressed per unit leaf area (A; Knipling, 1970; Terashima and Saeki, 1983; DeLucia et al., 1996). This ratio (A_{mes}/A) has also been strongly associated with photosynthetic performance in numerous species (Nobel, Zaragoza, and Smith, 1975; Sinclair, Goudriaan, and deWit, 1977; Longstreth, Bolanos, and Goddard, 1985).

Other characteristics of leaf structure that have been linked to changes in NIR reflectance were also investigated in the present study. For instance, Vogelmann and Martin (1993) showed that long, cylindrical palisade mesophyll (PM) cells propagate visible wavelengths deeper into the leaf interior, whereas the more spherical spongy mesophyll (SM) cells tend to scatter radiation. In general, SM may also have more cell wall–IAS interfaces that act to reflect light (Terashima and Saeki, 1983; DeLucia and Nelson, 1993). Thus, leaves with a greater PM/SM thickness ratio may also trap a greater amount of NIR radiation and have lower NIR reflectance values from the adaxial leaf surface.

Several factors other than cell wall–IAS interfaces may also contribute significantly to NIR reflectance from leaves. For instance, leaf pubescence in the desert species, *Encelia farinosa* and *Brickelia incana,* has been shown to increase NIR reflectance by up to 10% (Ehleringer, 1981), and epicuticular waxes on the leaf surface have also been shown to enhance NIR reflectance by 5–20% in the conifer tree *Picea pungens* and the succulent rosette *Dudleya brittonii* (Reicosky and Hanover, 1978; Mulroy, 1979). Thicker leaf cuticles may also lead to greater leaf reflectance of solar radiation (Gates, 1970) and removal of the lower epidermis of a bicolored leaf (abaxial surface a lighter shade of green than adaxial) reduced NIR reflectance from the adaxial leaf surface by up to 15% (Lin and Ehleringer, 1983).

The primary objective of the present research was to deter-

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TABLE 1. A list of species examined with family and growth form.

Species	Family	Growth form
1 *Antennaria umbrinella* Rydb.	Asteraceae	forb/mat
2 *Aquilegia caerulea* James	Ranunculaceae	forb
3 *Arctostaphylos uva-ursi* (L.) Spreng	Ericaceae	dwarf shrub
4 *Arenaria congesta* Nutt.	Caryophyllaceae	forb
5 *Besseya alpina* (Gray) Rydb.	Scrophulariaceae	forb
6 *Caltha leptosepala* DC.	Ranunculaceae	forb
7 *Campanula uniflora* L.	Campanulaceae	forb
8 *Carex nova* Bailey	Cyperaceae	graminoid
9 *Cerastium beeringianum* Cham. & Schlect.	Caryophyllaceae	forb
10 *Chionophila jamesii* Benth.	Scrophulariaceae	forb
11 *Deschampsia caespitosa* (L.) P. Beauv.	Poaceae	graminoid
12 *Erigeron compositus* Pursh	Asteraceae	forb
13 *Erigeron melanocephalus* Nels.	Asteraceae	forb
14 *Erigeron peregrinus* (Pursh) Greene	Asteraceae	forb
15 *Erysimum nivale* (Greene) Rydb.	Brassicaceae	forb
16 *Erythronium grandiflorum* Pursh	Liliaceae	forb
17 *Gentiana algida* Pall.	Gentianaceae	forb
18 *Gentiana parryi* Engelm.	Gentianaceae	forb
19 *Gentianella amarella* (L.) Boerner	Gentianaceae	forb
20 *Geum rossii* (R. Br.) Ser.	Rosaceae	forb/mat
21 *Haplopappus lyallii* Gray	Asteraceae	forb
22 *Hymenoxys grandiflora* (T. & G. ex Gray) Park.	Asteraceae	forb
23 *Kalmia microphylla* (Hook.) Heller	Ericaceae	dwarf shrub
24 *Lewisia pygmaea* (Gray) Robins.	Portulacaceae	forb
25 *Mertensia ciliata* (James ex Torrey) G. Don	Boraginaceae	forb
26 *Mertensia viridis* (A. Nels.) A. Nels.	Boraginaceae	forb
27 *Oxyria digyna* (L.) Hill	Polygonaceae	forb
28 *Pedicularis groenlandica* Retz.	Scrophulariaceae	forb
29 *Pedicularis parryi* Gray	Scrophulariaceae	forb
30 *Penstemon whippleanus* Gray	Scrophulariaceae	forb
31 *Phleum alpinum* L.	Poaceae	graminoid
32 *Poa nervosa* (Hook.) Vasey	Poaceae	graminoid
33 *Polygonum bistortoides* Pursh	Polygonaceae	forb
34 *Polygonum viviparum* L.	Polygonaceae	forb
35 *Potentilla concinna* Richardson	Rosaceae	forb
36 *Ranunculus alismaefolius* Geyer ex Benth.	Ranunculaceae	forb
37 *Salix glauca* L.	Salicaceae	dwarf shrub
38 *Senecio dimorphophyllus* Greene	Asteraceae	forb
39 *Senecio fremontii* T. & G.	Asteraceae	forb
40 *Sibbaldia procumbens* L.	Rosaceae	forb/mat
41 *Silene acaulis* (L.) Jacq.	Caryophyllaceae	cushion
42 *Solidago simplex* Kunth	Asteraceae	forb
43 *Thlaspi montanum* L.	Brassicaceae	forb
44 *Trifolium dasyphyllum* T. & G.	Fabaceae	mat
45 *Trollius laxus* Salisb.	Ranunculaceae	forb
46 *Vaccinium caespitosum* Michx.	Ericaceae	dwarf shrub
47 *Veronica wormskjoldii* R. & S.	Scrophulariaceae	forb
48 *Viola adunca* Smith	Violaceae	forb

mine whether leaf NIR reflectance at a single wavelength (NIRR; 800 nm) could be predicted quantitatively from a relatively simple model of leaf structural characteristics. Leaf structural parameters tested included the presence of leaf bicoloration and of a thick leaf cuticle (>1 μm), the degree of trichome density, leaf thickness, the PM/SM ratio, A_{mes}/A, and %IAS. The model was then tested using data from 48 species collected from an alpine region of southeastern Wyoming.

MATERIALS AND METHODS

Plant material—Leaf structure and reflectance were measured initially for six species (*Kalmia microphylla, Oxyria digyna, Phleum alpinum, Potentilla concinna, Thlaspi montanum,* and *Trifolium dasyphyllum*) that represent a broad variety of growth forms and family affiliations (Table 1). The plants were transplanted during July and August of 1997 from an alpine site in the Medicine Bow Mountains in southeastern Wyoming (106°19′ W, 41°20′ N) to the Williams Conservatory at the University of Wyoming. They were grown in soil transported from the alpine site and exposed to the natural photoperiod in a glasshouse with relative humidity maintained at ~40%, night temperature at ~6°C, and day temperature at ~22°C. A statistical model of leaf structure vs. reflectance was initially formulated using data from the six species grown in the glasshouse and then validated using data for leaves of 48 native alpine species (Table 1).

Leaf measurements—During October 1997, leaf structural characteristics and reflectance for the six species were measured for three individual leaves from each of three plants ($N = 9$ for each species). Light energy at 800 nm was measured from the adaxial surface of single, fresh leaves with a LI-1800 spectroradiometer (LI-COR, Inc., Lincoln, Nebraska, USA) and recorded as the average of three scans using a fiber optic probe with a 25° field of view. Leaves were illuminated with a 200-W quartz halogen light source. The probe tip was oriented in the nadir position (perpendicular to the leaf surface) and the light source was oriented at ~45° from nadir. Light energy was also

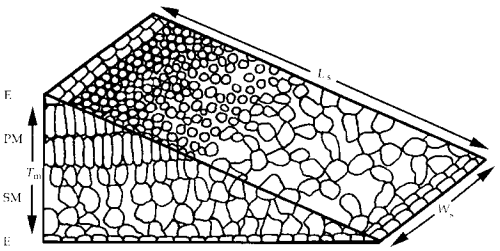

Fig. 1. A representative leaf section illustrating parameters for the calculation of A_{mes}/A and %IAS. The perimeter length of the mesophyll cells (P_i) and area of the intercellular air spaces, as viewed in the oblique-paradermal section, were measured to give A_{mes}/A and %IAS, respectively. See text for further explanation. (Modified from James, Smith, and Vogelmann, 1999.)

Figure Abbreviations: E, epidermis; L_s, length of oblique-paradermal section; PM, palisade mesophyll; SM, spongy mesophyll; T_m, mesophyll thickness; W_s, width of section.

measured for a white standard (Spectralon, Labsphere Inc., North Sutton, New Hampshire, USA), illuminated with the same orientation of the light source and probe. Bidirectional reflectance factors at 800 nm were calculated by dividing the values for light energy reflected off the leaf by those for the white standard. The leaf near-infrared reflectance (NIRR) was calculated by multiplying the bidirectional reflectance factor by 100 to give a percentage.

The presence or absence of leaf bicoloration was recorded for each leaf and was considered present when the two leaf sides were easily discernible as a lighter abaxial compared to darker adaxial surface. Adaxial surfaces of three leaves of each species were also inspected under a dissecting microscope to assign each species to one of three comparative categories of trichome density (0 = none or infrequent trichomes, 1 = scattered trichomes, 2 = dense, usually overlapping trichomes).

The presence of a thick leaf cuticle, leaf thickness, and PM and SM thicknesses were measured from transverse sections (3–4 μm thick) of embedded leaves using light microscopy. Embedding was necessary so that samples could be thin-sectioned and stored for measurements over ~1 yr. Comparisons with fresh sections indicated the embedding process did not alter the size, shape, or spacing of the mesophyll cells. Sections were cut midway along the length of the leaf, halfway between the midrib and the outer margin of the lamina, fixed in 3% glutaraldehyde in a 0.015 mol/L phosphate buffer (pH 6.9) under vacuum and dehydrated in a graded series of ethyl alcohol. The sections were then embedded in gelatin capsules using an acrylic resin (LR White, London Resin Co., Reading, UK), cut with glass blades on a microtome, and stained with 0.5% toluidine blue in 0.1% sodium carbonate buffer. All anatomical measurements were made using an ocular micrometer at three positions on each leaf sampled. If the mean thickness of the adaxial cuticle was >1 μm, it was scored as present. This cuticle thickness was chosen because it may be clearly detected using light microscopy and it enabled approximate equal division of the species examined into two categories of cuticle thickness.

A_{mes}/A and %IAS (% volume of mesophyll that was air space) were also measured for the embedded leaf sections, using the method described by James, Smith, and Vogelmann (1999). Oblique-paradermal sections (1 μm thick) were prepared as described above, but sliced at angles between 30° and 80° with respect to the plane of the adaxial epidermis (Fig. 1). Images of the sections were obtained with Image-Pro Plus software (Media Cybernetics, Silver Spring, Maryland, USA) using a video camera (Javelin Electronics, Los Angeles, California, USA) attached to a light microscope. These images were manipulated using Adobe Photoshop software (Adobe Systems, Inc., Mountain View, California, USA) so that contrast was maximized. The proportion of the mesophyll occupied by intercellular air spaces (%IAS) was calculated as the ratio of IAS area to the total area in the image (excluding the epidermises). All mesophyll cell surfaces exposed to IAS were traced and

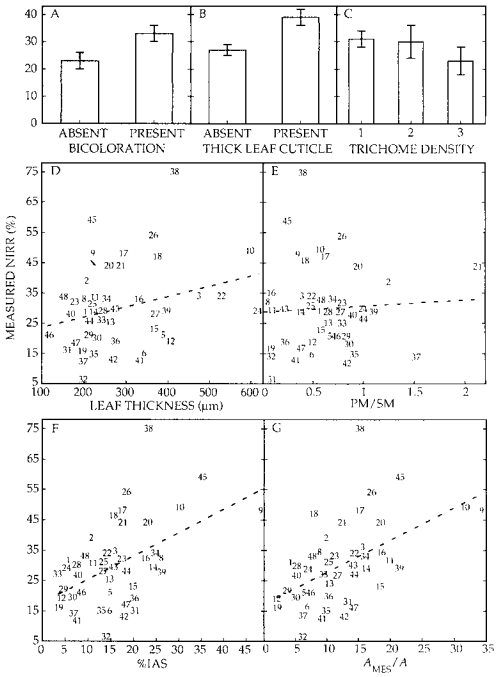

Fig. 2. Relationships between leaf structural characteristics and measured NIR reflectance at 800 nm (NIRR) in the 48-species data set. (A–C) Error bars indicate SE for these categorical variables (N = 48). (D–G) Fitted regression lines are shown for continuous leaf structural variables. Bicoloration and a thick leaf cuticle (>1 μm) were scored as either present or absent, and trichome density categories are 0 = none or infrequent trichomes, 1 = scattered trichomes, 2 = dense, usually overlapping trichomes. Regression analysis indicated NIRR was significantly correlated with the presence or absence of a thick leaf cuticle and bicoloration, %IAS, and A_{mes}/A ($P < 0.02$). See Table 1 for species labels (by number).

the trace lengths were summed to give P_i. The unitless parameter, A_{mes}/A, was then calculated as

$$A_{mes}/A = (P_i \times T_m)/(W_s \times L_s) \quad (1)$$

where T_m is the thickness of the mesophyll and W_s and L_s are the width and length of the oblique-paradermal section, respectively (Fig. 1; modified from James, Smith, and Vogelmann, 1999). One section from each of three leaves, selected randomly from the nine leaves examined per species, was used to calculate A_{mes}/A for the six-species data set. For the 48-species data set, the three leaves used for reflectance measurements for each species were also used to measure A_{mes}/A.

Statistical analysis—Simple and multiple linear regressions were used to evaluate the relationships between NIRR and leaf structure for the initial six species using Minitab software (Minitab Inc., State College, Pennsylvania, USA). Leaf reflectance at 800 nm (NIRR) was the dependent variable, and the presence of bicoloration and a thick leaf cuticle, the degree of trichome density, leaf thickness, the PM/SM ratio, A_{mes}/A, and %IAS were independent variables. Indicator variables were used in cases where regressions included categorical predictors (i.e., trichome density).

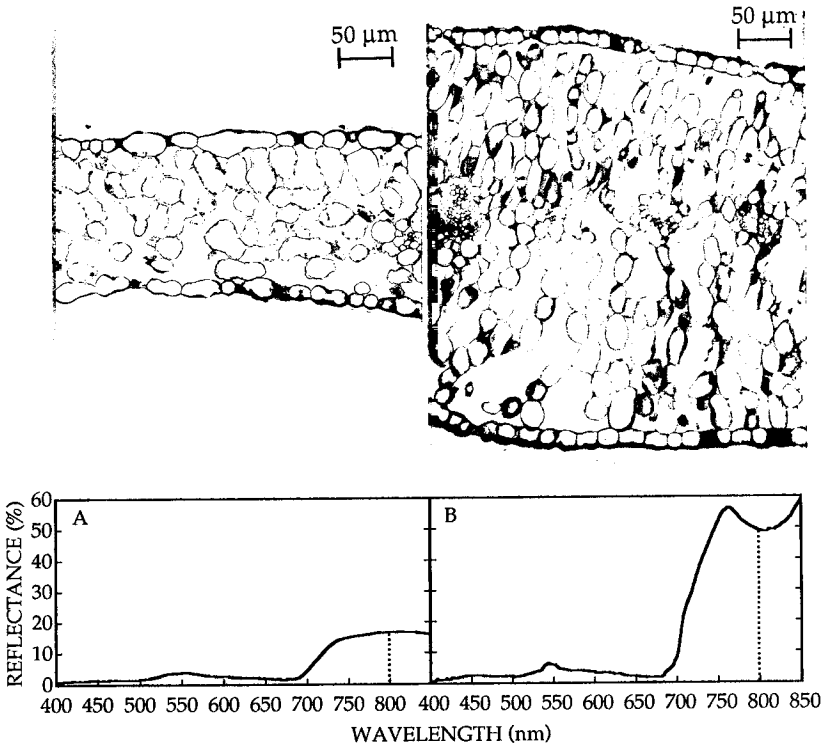

Fig. 3. Transverse sections and reflectance spectra from adaxial leaf surfaces for (A) *Gentianella amarella* and (B) *Chionophila jamesii*. *Gentianella amarella* and *C. jamesii* have relatively low (2.1) and high (31.7) A_{mes}/A values, respectively, for the 48 species investigated. Dashed lines indicate NIRR value (800 nm) used in the reflectance model.

Model validation—An empirical equation was developed for the six species grown in the glasshouse, in which NIRR was computed as a function of A_{mes}/A, leaf bicoloration, and cuticle thickness (Eq. 2, below). This model was then validated using leaf structure and reflectance data for leaves of 48 native species (Table 1). During July and August 1998, leaves from the 48 species were collected from the alpine field site and transported to the University of Wyoming on ice. Within 24 h, leaf reflectance was measured in the laboratory and leaf sections were embedded for structural measurements. Leaf bicoloration, cuticle, leaf thickness, and PM/SM data were collected from six leaves from each of six plants as described above ($N = 36$). For each species, NIRR, A_{mes}/A, and %IAS were measured for one healthy, mature leaf from each of three plants, selected randomly from the total of six plants examined per species.

RESULTS

Multiple regression analysis showed A_{mes}/A, leaf bicoloration, and the presence or absence of a thick leaf cuticle to be the three variables that most accurately predicted NIRR ($r^2 = 0.93$) in the original data set that included six species.

The regression equation was

$$\text{NIRR (\%)} = 6.5 + (0.8 \times A_{mes}/A) + (12.4 \times \text{bicoloration}) + (9.9 \times \text{cuticle}) \quad (2)$$

where bicoloration and cuticle were discrete values of zero or one, indicating the absence or presence of the characteristic, respectively. Regressions between all three predictors showed no evidence of intercorrelation among these variables ($r^2 < 0.05$). Bicoloration was the best single predictor of NIRR ($r^2 = 0.33$), whereas the best two-variable model included the presence or absence of bicoloration and a thick cuticle ($r^2 = 0.68$). Of all the leaf structural parameters examined, A_{mes}/A was the only variable ($P = 0.09$) that significantly improved a model containing only the presence or absence of bicoloration and a thick leaf cuticle. Simple linear regressions showed that NIRR was not strongly correlated with trichome density, leaf thickness, the PM/SM ratio, or %IAS ($r^2 < 0.25$; $P > 0.1$) in the six-species data set. Relationships between NIRR and leaf structural characteristics for the 48-species data set are illustrated in Fig. 2.

Representative reflectance spectra and transverse sections for *Chionophila jamesii* and *Gentianella amarella* are shown in Fig. 3. These two species had relatively high and low values for A_{mes}/A of 31.7 and 2.1, respectively, that were associated with strong differences in NIRR.

Computed values for NIRR using both Eq. 2 and data

TABLE 2. Measurements for leaf bicoloration and adaxial leaf cuticle with thickness >1 μm (0 = absent, 1 = present), A_{mes}/A, %IAS, leaf thickness (μm), and the palisade to spongy mesophyll thickness ratio (PM/SM). Values are means ± 1 SE. See Table 1 for species labels (by number).

Label	Bicoloration	Cuticle	A_{mes}/A	%IAS	Leaf thickness	PM/SM
1	0	0	4.7 ± 1.2	5.9 ± 2.6	214 ± 9	0.59 ± 0.03
2	1	1	9.9 ± 1.5	10.8 ± 2.5	207 ± 6	1.43 ± 0.12
3	1	1	15.3 ± 2.6	15.3 ± 2.2	475 ± 18	0.46 ± 0.03
4	0	0	11.9 ± 2.7	15.6 ± 4.0	267 ± 7	0.29 ± 0.04
5	1	0	6.4 ± 1.0	14.9 ± 2.5	389 ± 15	0.69 ± 0.05
6	1	0	6.6 ± 1.4	14.3 ± 2.1	344 ± 13	0.50 ± 0.03
7	1	1	12.8 ± 2.6	8.2 ± 0.6	221 ± 6	0.27 ± 0.05
8	1	1	8.0 ± 0.3	25.7 ± 1.7	201 ± 4	0.03 ± 0.02
9	1	0	34.3 ± 1.3	50.5 ± 7.8	222 ± 7	0.38 ± 0.04
10	1	1	31.7 ± 9.5	30.2 ± 3.4	598 ± 16	0.65 ± 0.07
11	0	0	19.8 ± 2.5	12.7 ± 1.9	222 ± 14	0
12	0	0	2.0 ± 1.3	4.2 ± 1.7	407 ± 15	0.56 ± 0.07
13	1	0	10.7 ± 3.3	14.0 ± 4.1	258 ± 6	0.74 ± 0.09
14	1	0	16.2 ± 1.1	24.6 ± 3.9	233 ± 5	0.42 ± 0.04
15	0	0	18.4 ± 4.5	19.9 ± 7.5	367 ± 10	0.68 ± 0.06
16	0	0	19.9 ± 2.3	23.3 ± 4.1	331 ± 17	0
17	1	1	15.2 ± 1.7	17.5 ± 2.5	294 ± 5	0.63 ± 0.03
18	1	1	7.9 ± 1.9	15.8 ± 2.3	375 ± 10	0.43 ± 0.04
19	1	1	2.1 ± 0.1	3.7 ± 0.6	194 ± 8	0.05 ± 0.02
20	1	0	18.5 ± 1.1	23.1 ± 5.1	259 ± 4	1.00 ± 0.06
21	0	1	12.5 ± 4.0	17.7 ± 3.5	288 ± 24	2.14 ± 0.17
22	1	0	14.4 ± 2.5	14.2 ± 3.6	530 ± 23	0.66 ± 0.13
23	1	1	10.9 ± 2.0	17.5 ± 2.2	179 ± 4	0.81 ± 0.04
24	0	0	4.4 ± 1.8	5.4 ± 1.2	619 ± 14	1.83 ± 0.39
25	1	0	10.3 ± 1.5	13.5 ± 1.8	218 ± 6	0.50 ± 0.02
26	1	1	17.1 ± 1.7	18.5 ± 2.3	366 ± 13	0.85 ± 0.08
27	1	0	11.7 ± 2.4	13.6 ± 4.0	370 ± 13	0.75 ± 0.05
28	1	0	5.1 ± 0.9	7.1 ± 1.7	246 ± 9	0.70 ± 0.06
29	1	0	3.4 ± 0.9	4.4 ± 1.3	212 ± 5	0.86 ± 0.04
30	1	0	4.9 ± 0.4	5.7 ± 0.4	231 ± 11	0.89 ± 0.09
31	0	0	13.4 ± 2.6	19.0 ± 3.1	161 ± 6	0
32	0	0	6.2 ± 1.7	14.0 ± 5.0	199 ± 6	0.02 ± 0.01
33	1	0	9.5 ± 3.5	3.3 ± 0.1	237 ± 8	0.86 ± 0.05
34	1	0	16.0 ± 1.0	24.8 ± 2.1	254 ± 8	0.67 ± 0.04
35	1	0	9.9 ± 1.5	13.5 ± 1.0	202 ± 5	0.92 ± 0.04
36	0	0	9.8 ± 2.3	20.2 ± 4.6	275 ± 8	0.23 ± 0.02
37	1	0	6.7 ± 1.7	6.9 ± 1.7	204 ± 4	1.80 ± 0.24
38	1	0	15.3 ± 2.6	23.3 ± 1.9	417 ± 40	0.47 ± 0.04
39	1	0	21.4 ± 3.5	25.5 ± 1.7	397 ± 10	1.32 ± 0.18
40	1	0	5.3 ± 1.2	7.1 ± 0.9	169 ± 3	0.95 ± 0.06
41	0	0	9.0 ± 0.8	7.6 ± 0.9	336 ± 7	0.33 ± 0.03
42	0	0	12.8 ± 1.4	18.0 ± 2.2	270 ± 6	1.02 ± 0.14
43	0	1	14.2 ± 4.4	14.0 ± 3.9	255 ± 20	0.21 ± 0.06
44	1	0	14.8 ± 3.6	18.5 ± 9.4	233 ± 7	1.07 ± 0.08
45	1	0	21.6 ± 1.4	35.4 ± 0.9	220 ± 5	0.25 ± 0.02
46	1	0	7.4 ± 1.8	8.6 ± 3.3	113 ± 5	0.78 ± 0.05
47	0	0	13.1 ± 1.8	18.4 ± 3.2	183 ± 4	0.40 ± 0.03
48	1	0	7.7 ± 2.0	9.3 ± 2.1	155 ± 4	0.55 ± 0.04

shown in Table 2 are plotted against measured NIRR values in Fig. 4 ($r^2 = 0.43$; $P < 0.01$). Two outliers were omitted from the plot (*Arenaria congesta* and *Campanula uniflora*) because their narrow leaf widths (<2 mm) were smaller than the field of view of the spectroradiometer's optical system. In addition, *Senecio dimorphophyllus* (labeled 38) had unusually high measured NIRR (75%), perhaps due to a particularly shiny cuticle and high specular reflectance. If data for *S. dimorphophyllus* were omitted, the r^2 value for the regression increased almost 21%. Two other outliers, *Cerastium beeringianum* and *Chionophila jamesii* (labeled 9 and 10, respectively, in Fig. 4) had high predicted NIRR values as a result

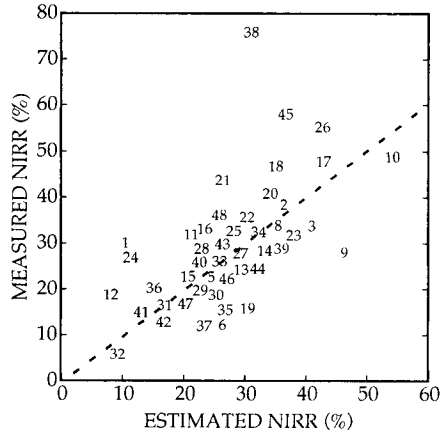

Fig. 4. Computed vs. measured NIRR (800 nm; $r^2 = 0.43$). Computed values were calculated from Eq. 2 (see Table 1 for species labels, by number). *Senecio dimorphophyllus* (labeled 38) had a particularly shiny cuticle which may have increased specular reflectance.

of unusually high A_{mes}/A ratios (34.3 and 31.7, respectively). Notably, there were no significant differences between the slopes ($t = -0.63$; $P = 0.53$) and intercepts ($t = 1.23$; $P = 0.23$) of the actual regression line, including outliers, and those of the line which indicated where predicted and measured values were equal.

DISCUSSION

The results presented here indicate that near-infrared leaf reflectance at 800 nm (NIRR) could be predicted accurately using an equation incorporating three parameters of leaf structure: (1) A_{mes}/A, (2) the presence or absence of leaf bicoloration, and (3) the presence or absence of a leaf cuticle thicker than 1 μm. A positive correlation between each of these parameters and NIRR was expected, based on previous evidence that they all may enhance reflectance of solar radiation from the adaxial leaf surface (e.g., Ehleringer, 1981; Lin and Ehleringer, 1983; DeLucia et al., 1996).

The absence, or weakness, of correlations between NIRR and other characteristics of leaf structure (trichome density, leaf thickness, the PM/SM ratio, and %IAS; Fig. 2) is notable. The weak correlation between reflectance and trichome density is in agreement with previous studies that found pubescence enhances NIR reflectance from leaf surfaces only slightly (by ~10%; Ehleringer, 1981). However, based on previous findings in the literature (e.g., Vogelmann and Martin, 1993), a significant correlation between the PM/SM ratio and leaf NIRR was expected, but not found. We hypothesized incorrectly that leaves with more PM would have lower NIRR from the adaxial leaf surface as a result of the greater propagation of radiation by the PM toward the leaf interior. However, this propagation property may be much stronger for visible wavelengths because it results, at least in part, from the sieve effect, where chloroplasts lining the cell walls of the PM create channels in the central vacuoles of the cells through which visible

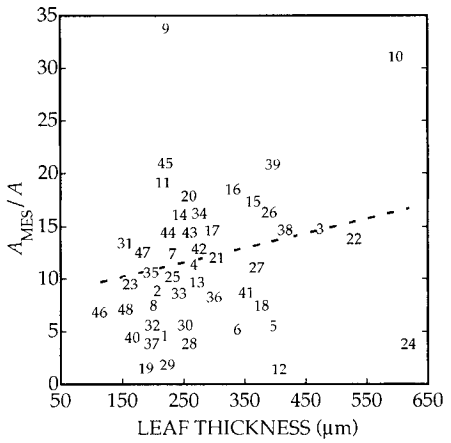

Fig. 5. A plot of leaf thickness vs. A_{mes}/A ($r^2 = 0.06$). The fitted regression line is indicated. See Table 1 for species labels (by number).

light passes without encountering chloroplasts (e.g., Fukshansky, 1981). This chloroplast distribution may not have such a strong effect on NIR vs. visible wavelengths due to strong absorption of visible light by chlorophyll.

The absence of a strong correlation between NIRR and leaf thickness found here is noteworthy (Fig. 2D). Gausman et al. (1973) also reported a weak association between greater leaf thickness and NIR reflectance in 20 crop species ($r^2 = 0.30$). In contrast, Ourcival, Joffre, and Rambal (1999) found a relatively strong correlation between these parameters in oak leaves. Knapp and Carter (1998) also found a strong correlation ($r^2 = 0.67$) between NIR reflectance and leaf thickness in 26 species representing a wide variety of growth forms. Leaf thickness has previously been shown to be correlated with A_{mes}/A (Chabot and Chabot, 1977; Smith and Nobel, 1977; Nobel, 1980; James, Smith, and Vogelmann, 1999). In such leaves, it is expected that leaf NIRR would be greater in thicker leaves that have more cell wall-IAS interfaces. However, in the present study, a weak correlation between A_{mes}/A and leaf thickness was observed (Fig. 5; $r^2 = 0.06$), and may account for the absence of a strong correlation between leaf thickness and leaf NIRR. Therefore, our data indicate A_{mes}/A may be a better predictor of NIRR than leaf thickness.

In addition, several species in the present study had relatively low A_{mes}/A values (<7) compared to those observed in different species by previous authors (A_{mes}/A = 9–77; Turrell, 1965; Longstreth, Hartsock, and Nobel, 1980). For other species with greater variation in leaf thickness and A_{mes}/A, a better correlation between NIRR and leaf thickness may exist. Leaf thickness and A_{mes}/A were not highly correlated in leaves in the present study due to variation in cell size and spacing. For example, *Cerastium beeringianum* (labeled 9 in Fig. 5) had small mesophyll cells (SM cell width <20 μm), while those of *Lewisia pygmaea* (labeled 24) were much larger (>45 μm). The mesophyll cells of *Trollius laxus* were widely spaced, with a high percentage of the cell surface area exposed to IAS, whereas the cells of *Hymenoxys grandiflora* and *Erigeron compositus* (labeled 45, 22, and 12, respectively) were more tightly packed.

A weak correlation between NIRR and %IAS was also observed here, for the six-species data set ($r^2 = 0.01$). Previous studies have found NIRR to be higher for more porous (high %IAS) leaves (Gausman, Allen, and Cardenas, 1969; Gausman et al., 1973). However, leaves with high %IAS in our original data set with six species did not necessarily have more exposed mesophyll cell surfaces where NIR radiation may be scattered. There was a relatively strong correlation between A_{mes}/A and %IAS ($r^2 = 0.71$), but the two parameters are not equivalent. The regression between %IAS and NIRR was statistically significant when 48 species were included ($r^2 = 0.26$; $P < 0.01$; Fig. 2F), although the correlation coefficient between A_{mes}/A and NIRR was greater ($r^2 = 0.29$; $P < 0.01$; Fig. 2G). Thus, our data indicate that A_{mes}/A, as opposed to %IAS, is a better estimator for leaf NIRR.

Conclusions—Leaf reflectance at a single wavelength in the NIR region (800 nm) could be estimated accurately from leaf structural characteristics in a group of 48 alpine species ($r^2 = 0.43$; $P < 0.01$). Leaves that had bicoloration, a thicker cuticle, and a higher proportion of mesophyll cell surface area exposed to intercellular air spaces per unit leaf surface area (A_{mes}/A) had predictably higher NIRR values from the adaxial leaf surface. Leaf trichome density, leaf thickness, and mesophyll proportion occupied by intercellular air spaces were not as effective predictors of NIRR in these species.

This relation between leaf structure and reflectance may be useful in the interpretation of remote sensing data measured from satellite or aircraft, or with standard field and laboratory instrumentation. For instance, because the presence of bicoloration and high values of A_{mes}/A may increase photosynthesis per unit leaf area (Nobel, Zaragoza, and Smith, 1975; Nobel and Walker, 1985; Smith et al., 1997), NIRR may be, for some species, a useful indicator of photosynthetic potential. However, the presence of thick cuticular wax may also reflect visible wavelengths, thereby reducing photosynthesis in certain species (Ehleringer, 1981). Thus, quantitative models relating leaf reflectance to structural characteristics may have important applications, including the estimation of photosynthetic potentials for different species via remote sensing of optical properties. Further investigation is required concerning techniques that may be used to relate these reflectance data for individual leaves to broader scales, such as an entire plant canopy.

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